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16. ABSTRACT

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MECHANISMS OF POLLUTANT-INDUCED AIRWAYS TOXICITY

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In a modern industrialized society it is virtually impossible to avoid exposure to at least low levels of respirable toxic substances.[36] Becausethescope of this problem has expanded greatly in recent years, a review of the toxicity of respirable compounds on airway function cannot be all inclusive. Therefore, this review will focus on certain areas.

First, data from epidemiologic and industrial studies suggest that early effects of respirable agents on airways may include: an increased frequency of bronchospasm in asthmatic subjects[17,84] and airway irritability in normal subjects;[34a,64,100a] and an increased incidence of airway infections in both normal subjects and subjects with pre-existing chronic obstructive pulmonary disease.[22a,40,52,54a,69,85] Thus, potential mechanisms that may lead to the evolution of these two specific abnormalities will be stressed.

Second, a review based on a simple division between "environmental pollutants" and "industrial" agents is arbitrary and probably no longer relevant for sub-acute exposures. For example, in the past, reactive aldehydes generally were encountered in an industrial setting.[35] However, with the increasing use of insulation in older homes and the increasing use of formaldehyde in building materials such as plywood and paneling, levels of aldehydes encountered in the atmosphere and in homes have risen even higher than the level considered safe in industry.[3,13] Therefore, in this review we have arbitrarily focused on a few classes of respirable toxic substances: (1) oxidant and irritant gases—O_3, and NO_2, and SO_2; (2) "particulate" materials—including the Na^+ and NH_4^+ salts of NO_3 and SO_4 compounds; (3) the reactive aldehydes—including formaldehyde and acrolein; and (4) heavy metals—including cadmium, nickel, zinc, and mercury.

Third, experimental studies that have been directed toward identifying mechanisms of toxin-induced airway dysfunction have been emphasized. However, because relatively few specific mechanisms have been described, this review is often speculative in nature and has been expanded where indicated to include "modes" of toxicity, i.e., sites or loci of damage.

AIRWAY IRRITABILITY OR HYPERREACTIVITY

Airway irritability or enhanced "reactivity" is a common response to airway injury.[12] Environmental exposure to oxidant gases, particularly O_3 and NO_2, increased the frequency of physician visits and hospital admissions for asthmatic subjects coincident with times of peak oxidant levels.[32,64] Similar data appear to link exposure to elevated levels of respirable particulate material in the atmosphere, particularly of compounds containing nitrate, to an increased incidence of asthmatic attacks.[25] Laboratory studies employing environmental chambers for controlled exposure to pollutants and measurements of airway reactivity to aerosols of histamine and/or methacholine have shown that relatively low levels of NO_2[66] and O_3,[31,38] but not nitrates,[80,96] induce airway reactivity in normal subjects. However, nitrates may induce airway responses during acute respira-

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tory infections.[96] Other compounds, such as nickel, have been shown to increase the incidence of bronchospasm in asthmatic subjects[63] and probably induce airway irritability in normal subjects, but have not been systematically studied in the laboratory.

Despite the relatively common expression of this mode of pollutant-induced airway dysfunction, its mechanisms are unknown. Several factors have contributed to this problem. First, as recently reviewed by Richardson[74] and Russell,[79] our understanding of human airway smooth muscle and its regulation is incomplete. Human airway smooth muscle has not yet been definitively characterized as a single unit or a multiunit system. Similarly, no integrated picture of the specific neural modulation or control of airway smooth muscle is available, although the roles of certain systems have been described. For example, it appears that constrictor tone to the airway wall is supplied by cholinergic vagal efferent fibers.[73] Vagal tone is probably modulated in part by a variety of afferent receptors in the airways although their quantitative contribution to vagal tone is unknown.

More difficult to evaluate has been the role of other neural systems in the regulation of airway smooth muscle relaxation. Both in vitro experiments with airway smooth muscle preparations and the in vivo response to exogenous beta-adrenergic agents suggest that there are beta receptors in human airway smooth muscle that induce a relaxation response.[73] However, in contrast to the presence of cholinergic efferent fibers, it appears from morphologic studies of human lungs that adrenergic neural innervation of smooth muscle is sparse, if existent at all.[75] Thus, beta receptors may be reactive primarily to circulating catecholamines.[73]

The relatively unimpressive evidence for adrenergic neural innervation of human airway smooth muscle led to a series of studies to characterize the role of a "nonadrenergic neural inhibitory system" in control of bronchomotor tone.[79] This neural system, which is analogous to the system that induces relaxation of the gut, has been identified by electrophysiologic studies in several mammalian airways and appears to induce smooth muscle relaxation.[25,26] The assessment of the role of this system in the regulation of airway smooth muscle tone has been difficult because the nature of a putative neurotransmitter remains controversial. The recent report that the neurotransmitter may be vasoactive intestinal peptide (VIP), if confirmed, should permit a better characterization of the role of this neural system in the control of human bronchomotor tone.[60] Finally, this general problem is complicated by the observation that, as is true of other airway functions, there appear to be substantial regional differences in control of airway smooth muscle between central and peripheral airways.[26]

Second, the pathogenesis of enhanced airway reactivity in the naturally occurring state, i.e., "asthma," is unknown.[55] Therefore, there are few clues upon which to focus in investigations of the nature of acquired hyperreactivity.

Third, as will be reviewed in detail below, the current approach to quantitating airway reactivity employing pulmonary mechanics and challenges with constrictor aerosols has been useful for the detection of early airways damage induced by toxic agents but has severe limitations with respectto identification of mechanisms. For example, pressure-flow data provide highly indirect measurements of airway diameter, complicated by problems of variable glottic aperture[98] and effects of lung volume history;[34] aerosol retention, distribution, and absorption are difficult to measure;[68] pharmacologic interventions, e.g., blockade, are seldom specific in humans or whole animal studies; finally, there is often an interdependence of effects, i.e., both pollutant and chemical agonists induce the same measured effect, airway constriction.

Despite these reservations, a number of interesting theories on mechanisms of toxin-induced airway reactivity have been advanced.

Pre-Existing Airway Constriction. A number of investigators have postulated that the enhanced airway reactivity measured as outlined above reflects increases in pulmonary resistance. Because airway flow resistance is inversely proportional to the fourth power of the airway radius, it is held that the enhanced response reflects the relatively large contribution of small but important reductions in baseline (i.e., pre-challenge with constrictor) airway caliber before and after intervention. Whether the reduction in airway diameter induced by the intervention is a function of increased bronchomotor tone or increased intraluminal products—mucus and/or cellular debris—is unclear and may vary with the toxic agent.

Several recent studies have specifically addressed this problem. Golden et al. studied O_3-induced airway hyperreactivity and were

careful to measure airway reactivity only after any exposure-induced changes in pulmonary resistance had returned to baseline.[31] Enhanced airway reactivity, with these constraints, was still readily demonstrable. Similarly, in asthmatic subjects Rubinfeld and Pain showed no correlation between baseline transpulmonary resistance and response to inhaled agents.[33] Therefore, whereas it is difficult to be absolutely confident that no baseline changes in airway diameter have occurred as a function of an experimental intervention, the recent evidence based on currently available tests of air flow resistance tend to discount this notion.

Abnormalities in Airway Smooth Muscle. Several different mechanisms may lead to the "sensitization" of airway smooth muscle by toxic substances, though data to support any of these mechanisms are limited. Possible mechanisms include the following: (1) Increased receptor sensitivity and/or receptor sites for acetylcholine. Although specific data relating this hypothesis to toxin-induced airway reactivity is lacking, Holtzman et al. have recently shown in atopic subjects that methacholine-induced airway hyperreactivity was not blocked by hexamethonium, a ganglionic blocking agent.[39] This would suggest that methacholine is acting in these subjects directly on airways smooth muscle via increased receptor affinity and/or number, or as discussed below, perhaps increased "access" of the agonist to smooth muscle rather than acting by a reflex mechanism.

(2) Conversion of the organization of smooth muscle. Assuming that airway smooth muscle normally behaves as a multiunit system, i.e., each muscle cell is specifically innervated,[44] multiunit systems may be converted into single unit or syncytial systems in response to lung injury. This conversion would result in the propagation of a single neural impulse to a number of muscle cells, enhancing or magnifying the constrictor response. Again, no evidence is available to support this hypothesis with respect to toxic exposure although multiunit systems can be converted into single unit system in vitro by exposure to tetra-ethyl ammonium.[43] (3) Tonic mediator release. It has been suggested that smooth muscle reactivity in asthma is a function of tonic local release of mediators from mast cells that act to "sensitize" muscle elements. The observation that non-immunologic mechanisms may have an important role in mast cell release have made this notion more attractive to account for pollutant effects on airway reactivity. Indeed, Menzel and co-workers have shown that incubation of rat peritoneal mast cells with ammonium mitrate (100 mM) will promote mast cell mediator release.[16] The in vivo demonstration of effects of agents at lower concentrations will be required, however, to better evaluate the relevance of this hypothesis. (4) Deficiency of nonadrenergic inhibitory system (NAIS) activity. It has been proposed that the NAIS may be deficient either in naturally occurring disease states, e.g., asthma, or may be damaged as a function of lung injury.[74] Finding a role for this mechanism in pollutant-induced airway dysfunction probably awaits a characterization of this system in normal control of bronchomotor tone and the demonstration that inhaled toxic substances may damage its function. (5) Beta blockage. It had been suggested that in asthma there may be a reduction in beta receptor activity.[92] Recently, a defect in cyclic-AMP generation in vitro in response to beta agonist exposure in airway smooth muscle from antigen-sensitive dogs has been reported.[77] While intriguing, no data from smooth muscle studies implicate this mechanism in pollutant-induced hyperreactivity.

Sensitized Afferent Vagal Neural Receptors. The concept of the "sensitized" vagal afferent receptor, developed in detail and reviewedby Widdicombe and Nadel,[61,102,103] suggests that as a function of airway damage, vagal afferent receptors, particularly intraepithelial "irritant receptors," become sensitized (i.e., have reduced firing thresholds) so that a given stimulus increases receptor activity and reflex bronchoconstriction. That the reflex effects are mediated by the efferent vagal system has typically been demonstrated by the ability to block the effects with inhaled or injected atropine or by vagal section or cooling.[54] Despite the initial appeal of this hypothesis, a number of recent observations are not consistent with it as originally proposed. For example, recordings of electrical activity in vagal afferent fibers from dogs in vivo has shown that acetylcholine does not directly stimulate afferent receptors.[97] Because the bronchoconstrictor cholinergic agent often used in inhalation challenge studies, methacholine, probably shares this characteristic with acetylcholine, it appears probable that this agonist must be working by mechanisms other than the stimulation of

"sensitized" vagal afferent fibers. As noted above, the data of Holtzman et al., employing hexamethonium to induce ganglionic blockade, also suggest that methacholine has a direct rather than reflex effect on airway smooth muscle.[39] Finally, as cited above, although ozone induces enhanced airway reactivity in dogs and humans,[18,44] direct recordings from vagal afferent fibers from dogs exposed to ozone suggested that vagal afferents are hyposensitive rather than hypersensitive to direct topical applications of histamine, rendering it unlikely that vagal afferents are mediating enhanced reactivity in this condition.[81] Because of these difficulties, the "sensitized" vagal afferent receptors has been expanded to include "increased access" of chemical agonists to receptors.[24] This revision more or less merges with the last hypothesis relating to these events, airway epithelial hyperpermeability.

Airway Epithelial Hyperpermeability. In this formulation, theairway epithelium forms the principal barrier that limits the movements of inhaled agents that are deposited on the airway surface to elements in the airway wall that control bronchomotor tone, i.e., neural afferents and/or smooth muscle.[8,37] Insults that damage the airway epithelium, principally the tight junctions adjoining airway epithelial cells, accelerate and increase the movement of chemical agonists deposited on the airway surfaces to effector sites in the airway wall, and by this mechanism enhance constriction.

A number of basic observations of the "barrier" function of the airway mucosa have contributed to this hypothesis. In particular the epithelium has been shown to act as the limiting barrier for solute and water translocation across airway surfaces. Small solutes, e.g., ions, may be transported at least partially through cellular pathways, either by traversing small pores (radius less than 2.8 Å) or by being translocated by carrier proteins.[95] Larger solutes, e.g., proteins that exceed the size of cellular pores, may move across epithelial barriers by transcellular routes, e.g., vesicles, or via paracellular routes. Recent observations have shown that the rate of movement of large molecules across airway surfaces in in vivo and in vitro studies suggest a relatively large equivalent pore (about ~50 to 100 Å) can be calculated for airway surfaces,[5,21] as contrasted to the relatively smaller equivalent pore (5 to 15 Å) reported from similar studies of alveolar surfaces.[93,94] Although these pores most likely reside in the region of "tight junctions," and hence the paracellular route probably dominates transepithelial protein movements, the precise morphologic correlates of "pores" have not been determined.

Little is known about the normal regulation of movement of these proteins across junctional sites because the precise structure and composition of the junction has not been described. Ultrastructural evidence from high resolution transmission electron micrographs suggest that tight junctions represent essentially fusions at sites betweeen adjacent cells. Freeze fracture investigations of these structures suggest that "fibrils" embedded in the cell membrane bi-layer serve to anchor adjacent cells to one another.[87] Although such fibrils have not been isolated from cell preparations or their composition described, several clues as to their nature have recently appeared. Cerreijido and co-workers have reported that the formation of tight junctions is dependent on protein synthesis.[15] Marin et al.[57] and Davis and co-workers[20] have reported data from in vitro studies suggesting that extracellular calcium is important for the maintenance of junctional integrity. Finally, the tight junctions become disorganized and proliferate when tracheal epithelium is maintained in organ cultures in vitro, suggesting that mechanisms exist in vivo for the control of synthesis and site of formation of junctional components.[14]

It appears that a variety of toxic insults damage the mucosal barrier. Damage is generally assessed by measuring rates of movements of probes across airway surfaces, comparing post exposure rates to control and/or demonstrating morphologic evidence of penetration of EM tracers, e.g., HRP, into normally protected spaces within the epithelium.[6,9] As an example of the latter approach, Figure 1 shows that inhaled cigarette smoke damages the epithelium, probably at the level of the tight junction, which allows HRP to flow from the lumen to the intercellular space. In general, because of technical difficulties with assessment of damage by probes or morphologic approaches, it is probably best to combine these techniques in the experimental animal for early detection of airway epithelial damage.[6,7,9]

Agents that have been reported to increase airway epithelial permeability are listed in Table 1. The diverse nature of the toxins that appear to damage the tight junction, and the

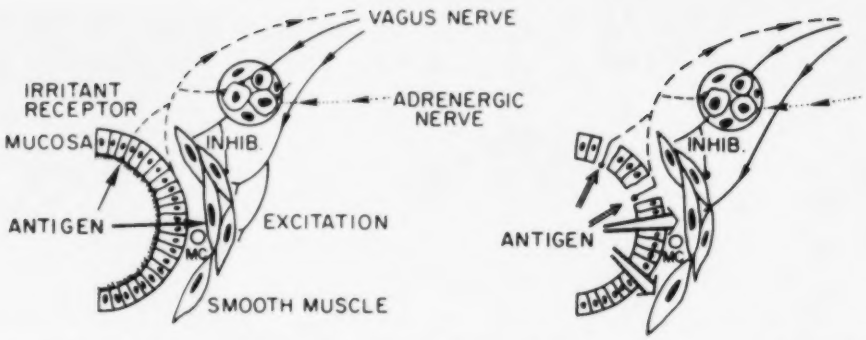

Figure 2. Left panel, normal epithelial barrier effectively limits antigen penetration to mast cell (MC)-rich submucosal spaces; *right panel,* hyperpermeable epithelium permits greater antigen penetration to submucosal spaces.

firmed and extended this observation by demonstrating that ozone exposure in guinea pigs increased airway epithelial permeability to a variety of large polar solutes, including HRP.[21] In addition, they showed that the hyperpermeability of the airway surface induced by ozone was associated with a loss of membrane restrictiveness, as estimated by enlargement of the epithelial pore radius. This finding would be compatible with damage to tight junctions and/or sloughing of epithelial cells. Figure 3 shows freeze-etch replicas of epithelial tight junctions from controls and animals exposed to ozone in these studies. Compared to controls, tight junctions examined from animals exposed to ozone showed derangement or even absence of fibrils, providing a morphologic correlate for an enlarged pore. These studies suggest that the effect measured by Matsumura was at least in part accounted for by raised airway epithelial permeability. Interestingly, in subsequent investigations Matsumura found enhanced pulmonary and systemic reactivity to aerosolized antigen in immunized guinea pigs after ozone exposure as compared to sham exposure.[59] This observation is compatible with the hypothesis that the enhanced immunologic reaction was induced by hyperpermeable airway surfaces allowing a greater antigen load to reach submucosal mast cells.

Similarly, circumstantial evidence is available to support the notion that toxin induced airway hyperreactivity to inhaled chemical agonists, e.g., histamine and methacholine, is related to mucosal hyperpermeability. For example, all the toxic agents listed in Table 1 that induce airway hyperpermeability have been shown in separate studies to induce airway hyperreactivity.[4,30,31,66] An opportunity to more specifically examine this relationship was afforded by the reports that acute allergic reactions in airways of Ascarsis-sensitive monkeys induced both mucosal hyperpermeability,[7] and hyperreactivity to cholinergic agents.[67] In a study designed to relate these two observations in the same animals, we measured in Ascaris-sensitive monkeys transpulmonary resistance (R_L) and the per cent of radiolabelled histamine absorbed into the systemic circulation after aerosol exposure to a standardized concentration of histamine.[8] Figure 4A shows that the monkeys demonstrated exaggerated R_L response to histamine aerosol after antigen challenge as compoared to sham exposure. Figure4B shows that fractional absorption of aerosolized radiolabelled histamine into the systemic circulation is significantly increased after antigen exposure as compared to controls, suggesting that airway hyperreactivity after specific antigen challenge is associated with increased permeation of histamine into the airway wall.

A problem with the hypothesis that airway epithelial hyperpermeability contributes to airway reactivity has been the repeated observations that histamine administered by the subcutaneous route is effective in eliciting airway hyperreactivity in ozone exposed guinea pigs.[23] Administration of the agonist by this route obviously bypasses any role for epithelial permeability in this phenomenon. However, because ozone has been shown to cause edema in a variety of small mammals,[83] and therefore likely alters capillary permeability,[70] this observation may be consistent with increased delivery of the drug to airway smooth muscle by means of capillary hyperpermeability.

In summary, it appears that the concept of airway hyperpermeability or increased access of inhaled agents or antigen to effector sites

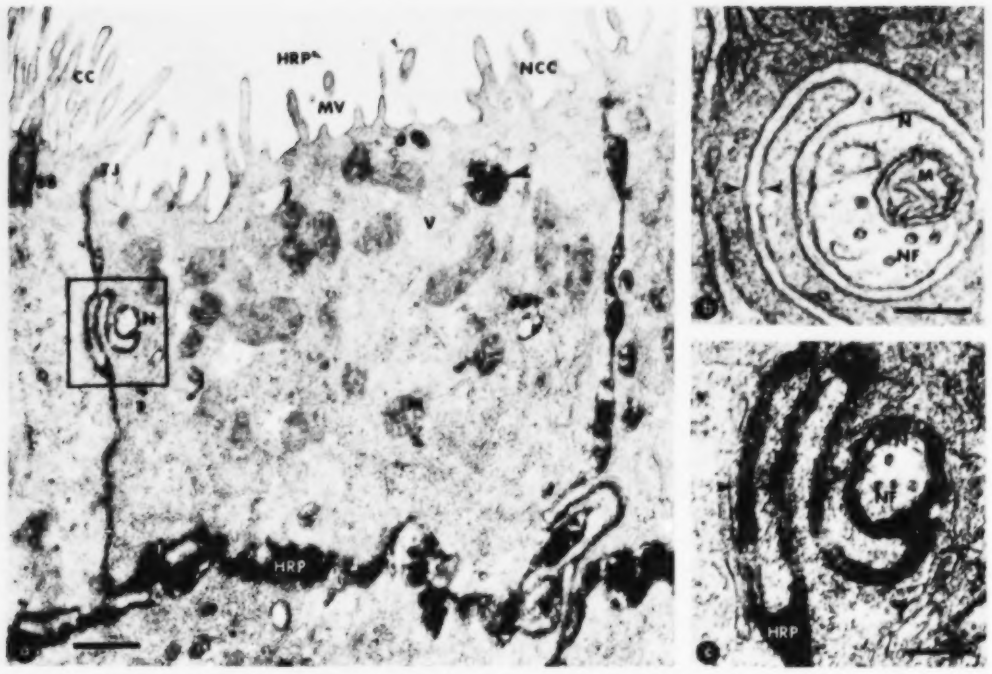

Figure 1. Section of guinea pig trachea exposed to cigarette smoke (100 puffs whole smoke). *A*, HRP reaction product noted on surface and intercellular spaces. *B*, Interepithelial neural structure, probably afferent, from control guinea pig. *C*, Higher powered view of area outlined in *A* showing HRP penetrating to locus of interepithelial neurons.

absence of a clear understanding of the nature of the junction, make it difficult to account for the damage to the junction induced by these agents by a unifying mechanism. In studies in guinea pigs systemic arterial hypoxemia or airway barotrauma did not appear to be likely mechanisms.[9] Intriguing potential mechanisms, all untested and not necessarily mutually exclusive, would include: (1) oxidant damage, either direct, by oxidant gases, or by free radicals derived from polymorphonuclear cells recruited to areas of damage; (2) enzymatic cleavage of junctions, either due to raised concentrations from circulating proteolytic factors or polymorphonuclear cells, or, perhaps, as reported in response to O_3 exposures, reduced local anti-protease activity;[42] and (3) metabolic changes within the cells themselves.

Table 1. *Agents That Increase Airway Epithelial Permeability as Measuredby In Vivo or In Vitro Techniques*

IN VIVO		IN VITRO
Toxic	*Allergic*	*Toxic*
O_3		
Type I Reactions		
Heavy metals		
NO_2	"Mediators"	Cadmium
SO_2	Histamine	Zinc
Cigarette smoke	Acethlcholine	Mercury
	? Type III/IV	Acid

It is epithelial damage, particularly within the tight junctions, that renders the mucosal surface hyperpermeable to, or "increases the access for," substances on the airway surface to the airway submucosal space. How this formulation can account for the increased incidence of attacks seen in asthmatic subjects after toxic exposure is shown in Figure 2. As compared to controls, an airway surface rendered hyperpermeable by toxin exposure allows accelerated flow of antigen, either inhaled or locally resident, across airway epithelial surfaces to the mast cell rich submucosal areas, triggering release of mediator and bronchoconstriction.

There is some evidence to support this notion. Matsumura and co-workers showed that guinea pigs exposed to ozone absorbed radiolabelled albumin more rapidly across pulmonary surfaces.[58] Davis and co-workers con-

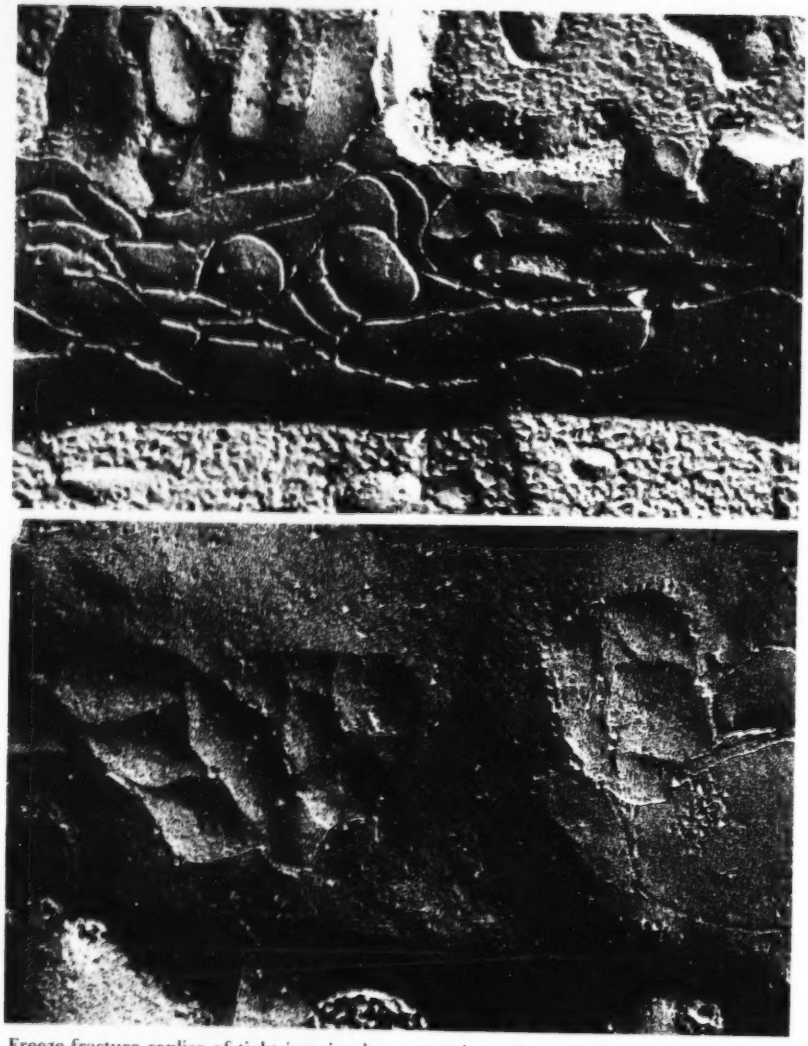

Figure 3. Freeze-fracture replica of tight junction between guinea pig tracheal epithelial cells. Top panel shows junctional configuration from control animal. Bottom panel shows junction from animal exposed to O_3 (4 ppm × 3 hrs). Arrow notes area of discontinuity of junction (both × 120,000). (Courtesy of Dr. J.L. Carson)

in airway walls is an attractive synthesis of many observations. However, at present whether or not hyperpermeability and airway hyperreactivity are casually related or merely associated is unknown.

MECHANISMS BY WHICH TOXIC SUBSTANCES INCREASE AIRWAY INFECTIONS

For obvious reasons no in vivo models comparable to the bronchoconstrictor inhalation challenge studies are available to assess pollutant related increases in lung infection in human subjects. However, studies of possible mechanisms by which pollutants may induce airway infection have been facilitated by a better understanding of normal airway defense mechanisms.[33,62,101] In particular, it has been recognized that airways are protected by a mucosal surface that exerts protective effects by (1) elaborating a complex mixture of glycoproteins and airway surface liquid that is continually cleared from the airway surface by ciliary motion and (2) forming a physical barrier composed of a confluent sheet of airway epithelial cells. The relation-

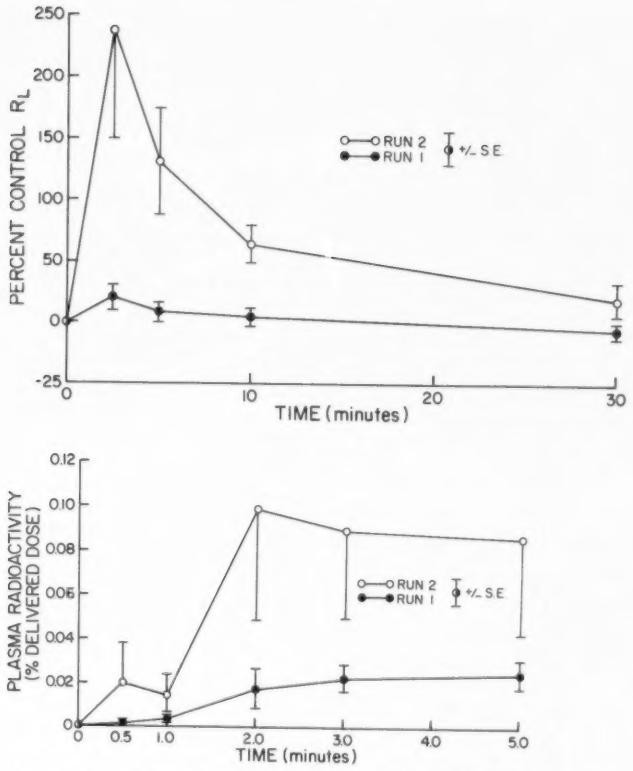

Figure 4. *Top,* Mean increase in airway resistance to histamine inhalations after sham (Run 1) and antigen challenges (Run 2); *Bottom,* Mean values for the accumulation of plasma radioactivity following initiation (time 0) of ^{3}H-histamine inhalations.

ship between abnormalities in mucociliary clearance and infection appears strong. For example, natural experiments, e.g., Kartagener's and subsequently a number of other "immotile cilia" syndromes, clearly demonstrate that impaired mucociliary clearance is associated with chronic pulmonary infection.[89] Therefore, because of the close relationship between mucociliary clearance and airways defense and the clues from environmental and experimental studies linking toxin-induced reductions in mucociliary clearance and infection,[99] much attention has focused on (1) the mucosal physiology that supports normal mucociliary clearance, and (2) loci and mechanisms of toxin-induced impairment of this system.

Elements of Mucociliary Clearance

The integrated function of at least three elements, i.e., "sol" or periciliary airway liquid; mucus "gel" or glycoprotein; and cilia, at both local and regional levels are necessary to maintain normal mucociliary clearance. Despite substantial gaps of understanding, considerable progress has been made in recent years in understanding the role of each of these elements.

For example, Kilburn stimulated interest in the regulation of the depth of the sol layer and the regulation of the overall volume and flow of airway surface liquid within the airways by the surface epithelium.[45] He pointed out that because liquid was cleared by ciliary activity from the large aggregate surface area of distal airways to the comparatively small surface area of central structures, airway surface liquid probably had to be absorbed before reaching the point of minimal airways cross-sectional area to avoid "drowning." As a corollary he postulated that the depth of the surface liquid had to be regulated locally by absorption to promote the close apposition of ciliary tip and "gel." Poor apposition of ciliary tip and gel, due either to raised or low-

ered depth of periciliary liquid, was postulated to result in decreased rates of mucociliary transport.

The initial attempts to directly measure electrolyte and water flow, i.e., components of "sol," across airway epithelium were reported by Olver and co-workers.[65] These studies employed the in vitro short circuiting (SC) techniques originally described by Ussing[50,95] to measure active ion transport across the excised posterior membrane of the canine trachea. In contrast to the prediction of Kilburn, these investigators found evidence under SC conditions for a relatively large Cl^- secretion toward the tracheal lumen and only a small component of Na^+ absorption. However, under open circuit (OC)—i.e., in experimental conditions more similar to in vivo conditions[4]—because the electrical gradient (PD) is not nulled by current passage—no net ion flows were detected. Recent preliminary work may help to resolve this issue. First, subsequent studies have identified in the trachea of several mammalian species, including dogs, a small but significant active absorption of Na^+ and perhaps bicarbonate in OC conditions.[11,19] These data suggest that at least in the resting state active absorption may be present in the trachea. Perhaps more importantly, in areas distal to the minimal cross-sectional area, i.e., in segmental and subsegmental bronchi, Na and Cl absorption have been detected.[11] These observations were made initially in canine bronchi but have been recently extended to other species, including in human bronchi.[49] Thus, it appears the depth of the bronchial surface liquid, both locally and axially along the airway, involves active ion absorption as originally postulated by Kilburn.

A model, incorporating these and other observations, to describe the net movement and volume control of airway surface liquid is shown in Figure 5. According to this schema, an isotonic solution is formed distally, either by alveolar surfaces or small airways. This surface liquid is swept toward more central surfaces by the effects of ciliary motion and perhaps physical forces. As the solution moves more centrally, mucin glyco-

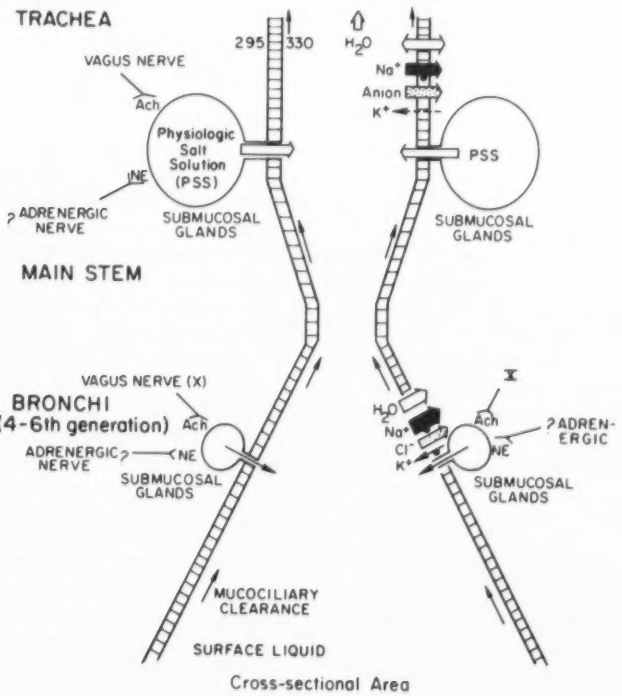

Figure 5. Schematic representation of airway cross-sectional area. Active ion translocation (arrow with appended circle), passive movement (arrow), and relative magnitude of net movements across surface epithelial cells noted. Osmolality of tracheal surface liquid and projected water flows in various regions shown. Projections of glandular secretion and regulation noted.

proteins are added by goblet cells and by glands that are likely to be at least in part regulated by neural mechanisms. In segmental bronchi, it appears that K^+ and perhaps HCO_3^- may be added to the luminal liquid. Thus, the overall volume and composition of the liquid on the airway surface at any locus reflects the interaction of several processes including: (1) the generation of transepithelial salt concentration gradients by active ion transport mechanisms; (2) the passive transepithelial flow of salt and water; (3) the central movement of surface liquid via ciliary motion; and (4) in some regions, e.g., trachea, evaporation of surface liquid into incompletely conditioned inspired air.[10,22,56]

Knowledge of the other elements of normal mucociliary clearance, i.e., the characterization of ciliary activity, and the quantity, composition, and visoelastic properties of the gel phase or mucus glycoprotein is also fragmentary. For example, little is known about the factors that normally regulate and coordinate ciliary activity in mammalian airways because measurement techniques have until recently been relatively crude and yield only semiquantitative data on beat frequency.[82] Perhaps a more fundamental problem has been that most measurement techniques have required in vitro approaches, without adequate in vivo measurements for comparison or studies of in vivo regulation directly.[104] Similarly, past efforts to measure concentrations of mucus on airway surfaces in different regions and efforts to partition quantitatively the contribution of the mucin secreting elements, i.e., glands and goblet cells, have been hampered by difficulties in measurements of microquantities of mucus. Microtechniques, however, have been developed to measure and describe the viscoelastic properties of normal mucus.[48] These studies by King and co-workers suggest that there may be a relatively narrow optimum in mucus viscoelastic properties that will support normal transport rates.[47,48]

Effects of Toxic Substances of Elements on Mucociliary Clearance

Abnormalities in Regulation of Volume and Composition of the "Sol" Layer. Recent evidence from in vitro studies of airway epithelia excised and mounted in Ussing chambers suggest that a number of airborne compounds may exert toxic effects on airway epithelial transport activities. A number of different mechanisms of action by these substances on airway epithelial function have been suggested in these studies.

The effects of ammonium and sodium salts of nitrate and nitrite compounds on the bioelectric properties and unidirectional ion fluxes of Na^+ and Cl^- across excised canine trachea have been studied.[91] Sodium salts of nitrite and nitrate (up to 100 mM) had little effect. However, ammonium nitrate or ammonium Cl^- (100 mM) reduced short circuit current (Isc), Na^+ absorption, and Cl^- secretion. Exposure of the luminal surface of the tracheal membrane led to an approximate 50 per cent reduction in Isc and the transepithelial electric potential differences (PD); submucosal exposure completely abolished these parameters. The effects were rapidly reversible by washing the tissue with fresh ammonium-free solutions. The use of bicarbonate-free (Hepes buffered) solutions potentiated the effects of ammonium on tracheal bioelectric and ion transport properties. The observations are consistent with the hypothesis that the dissolved NH_3 in NH_4^+ salt solutions penetrates the cell membrane, alkalinizes the epithelial cell cytoplasm, and blunts transport. Whereas further measurements are necessary to confirm this hypothetical mechanism, it is intriguing to speculate that a number of airborne toxic substances may exert toxic effects by alterations in intracellular pH.

The effects of in vitro heavy metal exposure on airway epithelial bioelectric properties have been reported using the excised canine trachea as a model system for airway ion transport.[91] Luminal exposure to cadmium salts induced dose-dependent (to 3×10^{-3}M) reductions in Isc and increased membrane conductance (G). Luminal exposures to zinc salts (to 3×10^{-3}M) was without effect. In addition, luminal exposure to cadmium in the presence of zinc produced a reduction in Isc but no change in G. Thus, in canine trachea Zn may block the access of cadmium to sites on the luminal surface that are associated with transmural conductance but afforded no protection to the presumably cellular loci that mediate the cadmium induced reduction in short circuit current.

Studies of mercury (Hg) toxicity, employing the bullfrog lung as a model of alveolar ion transport function have been reported.[28] Low concentrations of Hg (10^{-7}M) increased Isc whereas, at higher concentrations (10^{-5}M) Isc was inhibited and conductance

raised. Further, at these higher concentrations, Hg appeared to reduce oxygen consumption. Thus, it appears that Hg effects at higher concentration may be mediated by cellular metabolic depression.

Only very preliminary data are available with respect to the effect of reactive aldehydes on airway epithelial functions. Formaldehyde (1 mM) reduces Isc and G when added to the luminal surface of canine bronchi. Acetaldehyde demonstrated similar effects at higher concentrations (10 mM). This pattern of response suggests that these agents may affect the entry of sodium into the cell at the luminal barrier. However, further investigations of this potential mechanism are needed.

The effects of ammonium and sodium salts of nitrate and nitrite compounds on the bioelectric properties and unidirectional ion been explored (unpublished observations). In these studies the pH of the solution bathing the luminal surface of the canine trachea was changed by replacing the normal bathing solution with an unbuffered Ringer solution titrated to defined pH values. It was found (Figure 6) that bioelectric properties were not altered until the pH of the luminal bathing solution approached 2.8. Luminal solution pH values in the 2.2 to 2.8 range produced rapid, large drops in transepithelial PD that reflected primarily an increase in membrane conductance. These changes were rapidly reversible by returning the pH of the luminal solution to 7.4. When the luminal pH was 2.2 or less, short circuit current dropped, PD approached 0, and these changes were irreversible. The submucosal side of the canine trachea was more sensitive to proton addition. Solution pH values of 5.0 or leses induced irreversible changes in short circuit current and conductance. All acids induced identical effects, suggesting the proton concentration was responsible for these changes.

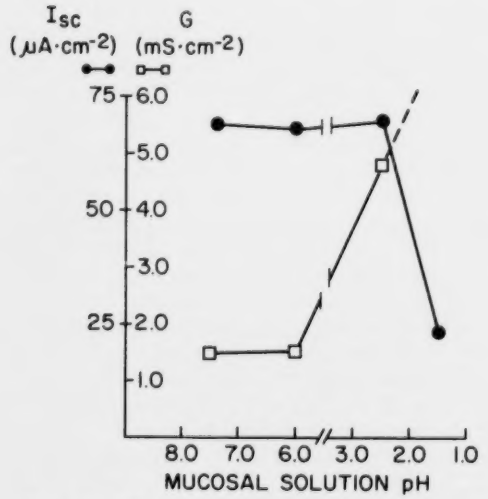

Figure 6. Effect of mucosal solution pH in canine tracheal epithelial bioelectric properties.

Gatzy and Stutts employed a similar approach with bullfrog lung and reported that exposure of the luminal surface to a pH of 2.5 increased G several fold.[29] In addition, measurements of ^{14}C-mannitol permeability coefficients showed that the acid-induced increase in G was accompaniedby greatly accelerated rates of movement of this probe, implying increased movement through paracellular routes. Comparison of the rates of mannitol movement to another solute (Cl^-) to estimate an equivalent "pore" radius suggested that acid induced an enlargement of the "normal" pores in the bullfrog from 15 Å to > 40 Å. Thus, it appears that at intermediate pH values (2.5) that protons may exert effects on tight junctions, perhaps loosening the adherence of adjacent junctional fibrils, and increasing equivalent pore radii. The potential contribution of this mechanism of toxicity to inhaled mineral acids awaits measurements of airway surface pH in response to in vivo exposures.

Besides the relationship between the "sol" phase and mucociliary clearance, an unexplored relationship between toxin-sol interactions and infection may relate to local surface liquid pH. Recent data[10] suggest the pH of bronchial surface liquid may be alkaline (pH 7.8 to 8.2). If bacterial attachment to bronchial surfaces is dependent on pH, an alkaline pH may exhibit attachment and serve as a "protective" mechanism. Hence, abnormalities in sol pH, either directly induced for example, by acid deposition, or indirectly induced by alterations in active ion transport mechanisms, in certain circumstances may promote bacterial attachment and subsequently infection.

Finally, as discussed previously, a number of agents have been shown to increase solute permeation through epithelial tight junctions in vivo. As small ions traversae this pathway as well, these increases in permeability would be expected to increase membrane conductance when measured in vitro electrophysiologically. However, to date the effects of ox-

idant injury on airway epithelial function measured in vitro have not been reported.

Ciliary Toxicity. Frank loss of cilia has been observed after exposure to high concentrations of various toxic substances, including SO_2, NO_2, and O_3.[18,88,100] These and other agents, e.g., acrolein, at lower concentrations, have been shown to cause abnormalities in ciliary beat frequency.[18] Collection of data on the specific mechanisms of toxicity of these agents has been hampered both by the poor understanding of the normal regulation of mammalian ciliary beat frequency in vivo and the insensitivity of the measurement techniques. However, certain clues have become available from recent studies employing both physiologic and morphologic approaches.

Gardner and co-workers reported that cadmium salts (Cd) in vitro (to 10^{-6}M) and in vivo induced reductions in ciliary beat frequency.[98] These authors speculated that Cd penetrated the intracellular space and inhibited ATPase activity committed to ciliomotion. Data consistent with this mechanism was reported by Gatzy in studies on the motility of the cilia that reside at the tips of the trabecular ridges of the amphibian lung.[27] Initially, he found that mercury at relatively low concentrations (10^{-6}M) and nickel and cadmium salts at relatively high concentrations (10^{-4}M) induced significant depressions in ciliary motility. Then he showed that sulfhydryl compounds that permeate into cellular spaces, e.g., mercaptoethanol and cysteamine, reversed the inhibitory effects of cadmium. Sulfhydryl groups on molecules that are restricted to the extracellular space, e.g., albumin, hemoglobin, or dithiothreitol, were ineffective in reversing ciliostasis. These results suggested that cadmium inhibits ciliomotion by interaction with intracellular ligands—including possibly the ATPase as postulated by Gardner. Effects of toxic agents on intracellular Ca^{++}, probably a critical variable in normal ciliomotion,[82] have not been explored.

Quantitative morphologic data on the effect of exposure to toxic substances in cilia in experimental animals have recently appeared. Ranga and Kleinerman[71] have shown that exposure of mice to NO_1 (20 ppm for 28 days) induced abnormalities in bronchiolar cilia that were characterized by: (1) decreased number of cilia per cell surface; (2) local swelling of the ciliary shaft; and (3) compound cilia (Fig. 7). These abnormalities appeared to be localized to the ciliary shaft because the ciliary basal bodies and other intracytoplasmic organelles in ciliated cells appeared normal. The cilia, at least morphologically, returned to normal within 30 days after cessation of the exposure. The mechanism of action of this presumed oxidant-induced injury of the ciliary shaft is unknown although it is intriguing to speculate that NO_2 may have induced local abnormalities in ion permeabilities in the membrane of the ciliary shaft that produced the "swelling."

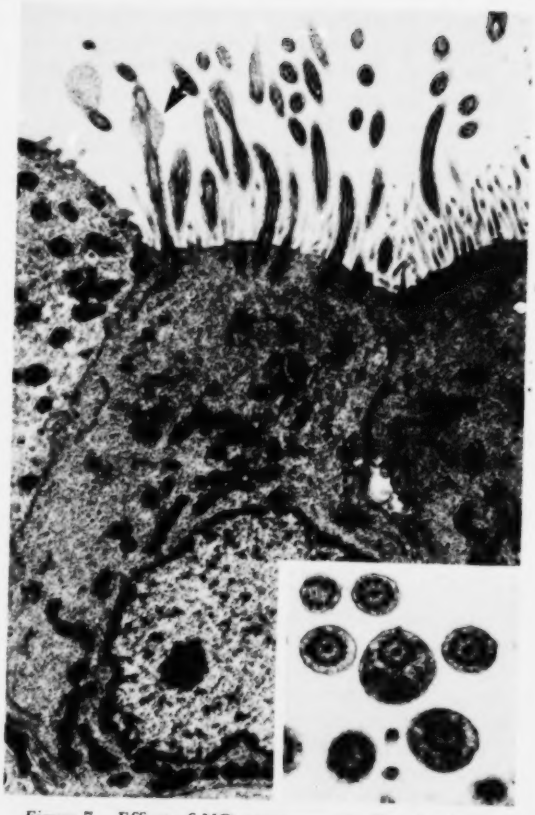

Figure 7. Effect of NO_2 exposure on cilia of guinea pig bronchiolar epithelium (×7000). Arrow notes swelling of ciliary shaft. Cross-sectional view of ciliary swelling shown in inset (×22000). (Courtesy Drs. V. Ranga and J. Kleinerman.)

Glycoproteins. The gel phase or glycoprotein component of lung surface liquid might contribute to reductions in mucociliary transport by at least two mechanisms. First, Dalham showed that excess secretion of glycoproteins appeared to retard transport.[18] Secondly, in vitro studies have correlated abnormalities in viscoelastic properties of mucus with reduction in transport.[48]

Morphologic studies have suggested mucus secretion can be increased by toxic exposure. SO_2, NO_2, as well as tobacco smoke exposure induce goblet cell hyperplasia and occasionally glandular hypertrophy in experimental animals, suggesting a possible source of excess secretions.[51,72] Shifts in the staining characteristics of intracellular glycoproteins, from neutral to acidic species, has also been reported in morphologic studies.[72] Last and coworkers have attempted to directly quantitate pollutant effects on glycoprotein secretion using tracheal explant systems.[53] First, they showed that acute exposure of rats to O_3 appears to decrease glycoprotein secretion. However, after two weeks of O_3 exposure (0.8 ppm) in rats [one week in monkeys (0.8 ppm)], glycoprotein secretion rates increased. Preliminary biochemical evidence also was presented for a shift in the nature of the glycoprotein secreted from neutral to acidic fractions. Although these studies in general appear to agree with in vivo morphologic studies, key issues remain. Specifically, what are the mechanisms that control the early depression and subsequent increase in secretion rates? How and to what degree are these changes in secretion rates partitioned between glands and goblet cells? What are the stimuli for goblet cell/gland hyperplasia?

King and co-workers have examined the effects in chronic SO_2 exposure (600 ppm) on viscoelastic properties of canine mucus.[46] They found that mucus sampled from exposed animals showed small reductions in elasticity and more substantial reductions in viscosity, effectively reducing the viscosity/elasticity ratio. These changes would be predicted to increase transportability and a trend in this direction was observed in transport studies employing a frog palate assay. If a specific abnormality in glycoprotein secretion from neutral to more acidic forms occurred in this model, as has occurred in other models of SO_2 exposure, it is tempting to speculate that this shift may constitute an adaptive response to facilitate mucociliary transport rather than confer disadvantageous results.

SUMMARY

The paper has focused on interactions of inhaled agents with airway surfaces. The respiratory mucosa is directly exposed to relatively high concentrations of inhaled substances and pollutant-related increase in incidence of infections and bronchospasm may reflect dysfunction of this barrier. Although the description of the mechanisms of toxicity to this surface are preliminary, it is clear that the challenge for the future will be to identify such toxic mechanisms. Too many environmental compounds are of concern and too many protocols are required to thoroughly study the toxicology and human health effects of individual compounds. An understanding of mechanisms is needed to categorize these compounds toxicologically to predict interactions between compounds in their effects on airways, and perhaps, to deal with the genetic variability of response demonstrated by individual subjects. A challenging need in future research will be to utilize observations on mechanisms made in animal models and in vitro to design well-focused experiments with human subjects.

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REFERENCES

1. Adalis, D., Gardner, D. E., Miller, F.J.: Toxic effects of cadmium on ciliary activity using a tracheal ring model. Environ. Res., *13*:111–120, 1977.
2. Austen, K. F.: Review of immunologic, biochemical and pharmacologic mechanisms in the release of chemical mediators from the human lung. *In* Austen, K. F., and Lichtenstein, L. M., eds.: Asthma: Physiology, Immunopharmacology and Treatment. New York, Academic Press, 1973.
3. Bardana, E. J.: Formaldehyde: Hypersensitivity and irritant reactions at work and in the home. Immunol. Allergy Prac., *60*:11–23, 1980.
4. Boileau, R., Delaunois, L., Trenblay, J., et al.: Enhancement of bronchial responsiveness to methacholine during chronic SO_2 exposure. Fed. Proc., *38*:1110, 1979.

4a. Boucher, R. C., Bromberg, P. A., and Gatzy, J. T.: Airways transepithelial electric potential in vivo: species and regional differences. J. Appl. Physiol., *48*:169–176, 1980.

5. Boucher, R. C., and Gatzy, J. T.: Tracheal epithelial permeability to non electrolytes. Physiologist, *21*(4):11, 1978.
6. Boucher, R. C., Johnson, J., Hunt, W., et al.: Effects of graded doses of cigarette smoke on the guinea pig trachea. Lab. Invest., *43*:94–100, 1980.
7. Boucher, R. C., Pare, P. D., Gilmore, N. J., et al.: Airway mucosal permeability in the ascaris-sulum sensitive Rhesus monkey. J. Allergy Clin. Immunol., *60*:134–140, 1977.
8. Boucher, R. C., Pare, P. D., and Hogg, J. C.: Relationship between airway hyperreactivity and hy-

perpermeability in ascaris sensitive monkeys. J. Allergy Clin. Immunol., *64*:197–201, 1979.
9. Boucher, R. C., Ranga, V., Pare, P. D., et al.: The effect of histamine and methacholine on guinea pig tracheal permeability to HRP. J. Appl. Physiol., *45*:939–948, 1978.
10. Boucher, R. C., Stutts, M. J., Bromberg, P. A., et al.: Regional differences in composition of airway surface liquid. J. Appl. Physiol.; Resp. Env. Exe. Physiol., *50*:613–620, 1981.
11. Boucher, R. C., Stutts, M. J., and Gatzy, J. T.: Regional differences in canine airway epithelial ion transport. Submitted for publication.
12. Boushey, H. A., Holtzman, M. J., Sheller, J. R., et al.: Bronchial hyperactivity. Am. Rev. Resp. Dis., *121*:389–413, 1980.
13. Breusse, P.: Formaldehyde in mobile and confentional homes. Environmental Health and Safety News, *26*:1–20, 1977.
14. Carson, J. L., Collier, A. M., and Hu, S. S.: Ultrastructural changes of hamster tracheal epithelium *in vivo* and *in vitro* J. Ultrastruc. Res., *70*:70–81, 1980.
15. Cereijido, M., Robbins, E. S., Dolan, W. L. J., et al.: J. Cell Biol.,*77*:853–880, 1978.
16. Charles, J. M., and Menzel, D. B.: Ammonium and sulfate ion release of histamine from lung fragments. Arch. Environ. Health, *30*:314, 1975.
17. Cohen, A. A., Bromberg, S., Buechley, R. W., et al.: Asthma and air pollution from a coal-fueled power plant. Amer. J. Publ. Health, *62*:1181–1188, 1972.
18. Dalhman, T.: Mucous flow and ciliary activity in the trachea of healthy rats and rats exposed to respiratory irritant gases (SO_2, H_3N and HCHO). Acta Physiol. Scand., *36*:1–161, 1956.
19. Davis, J. D., Boucher, R. C., Gatzy, J. T., et al.: Pattern of salt transport in excised rabbit trachea. Physiologist,*23*:100, 1980.
20. Davis, B., and Nadel, J. A.: New Methods used to Investigate the control of Mucus Secretion and Ion Transport in Airways. Environ. Health. Perspec., *35*:121–130, 1980.
21. Davis, J., Daniel, J., Gallo, E. P. C., et al.: The effects of ozone on respiratory epithelial permeability. Amer. Rev. Resp. Dis., *121*:231, 1980.
22. Deal, E. C., Jr., McFadden, E. R., Jr., Ingram, R. H., Jr., et al.: Role of respiratory heat exchange in production of exercise induced asthma. J. Appl. Physiol., *46*:476, 1979.
22a. Douglas, J. W. B., and Waller, R. E.: Air pollution and respiratory infection in children. Brit. J. Prev. Soc. Med., 20:1, 1966.
23. Easton, R. E., and Murphy, S. D.: Experimental ozone pre-exposure and histamine. Arch. Environ. Health, *15*:160–166, 1967.
24. Empey, D. W.,Latinen, L. A., Jacobs, L., et al.: Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. Am. Rev. Resp. Dis., *113*:131–139, 1976.
25. EPA Summary Report on Atmospheric Nitrates: U.S. Environmental Protection Agency National Environmental Research Center, Research Triangle Park, North Carolina, 1974.
26. Fleish, J. H., and Calkins, P. J.: Comparison of drug-induced responses of rabbit trachea and bronchus. J. Appl. Physiol., *41*:62–66, 1976.
27. Gatzy, J. T.: Cd binding and functions of alveolar cells disaggregated bullfrog lung. Fed. Proc., *37*:808, 1978.
28. Gatzy, J. T.: Heavy metals and membrane functions of an elveolar epithelium. *In* Shamoo, A., and Miller, M., eds.: IX international Rochester toxicity conference on membrane toxicity, N.Y., Plenum, 1977.
29. Gatzy, J. T., and Stutts, M. J.: Chemical modulation of alveolar epithelial permeability. Environ. Health Perspec., *35*:13–20, 1980.
30. Gerrard, J.W., Cockroft, D. W., Mink, J. T., et al.: Increased nonspecific bronchial reactivity in cigarette smokers with normal lung function. Am. Rev. Resp. Dis., *122*:577–582, 1980.
31. Golden, J. A., Nadel, J.A., and Boushey, H. A.: Bronchial hyperreactivity in healthy subjects after exposure to ozone. Am. Rev. Resp. Dis., *118*:287–294, 1978.
32. Gray, E., MacNamee, J. K., and Goldberg, S. B.: Toxicity of NO_2 vapors at very low levels. Arch. Industr. Hyg., *6*:20, 1952.
33. Green, G. M., Jakab, J., Low, R. B., et al.: Defense mechanisms of the respiratory membrane. Am. Rev. Resp. Dis., *115*:479–514, 1977.
34. Green, M., Mead, J.: Time dependence of flow-volume curves. J. Appl. Physiol., *37*:793–798, 1974.
34a. Hammer, D. I., Hasselblad, V., Portnoy, B., et al.: Los Angeles student nurse study. Daily symptom reporting and photochemical oxidants. Arch. Environ. Health, *28*:255–260, 1974.
35. Hendrick, D. J., and Lane, D. J.: Occupational formalin asthma. Br. J. Indust. Med., *34*:11–12, 1977.
36. Hinds, W. C. The lung and the environment. Seminars Resp. Med.,*1*:197–207, 1980.
37. Hogg, J. C., Pare, P. D., and Boucher, R. C.: Bronchial mucosal permeability. Fed. Proc., *38*:197–201, 1979.
38. Holtzman, M. J., Cunningham, J. H., Sheller, J. R., et al.: Effect of ozone on bronchial reactivity in atopic and non-atopic subjects. Am. Rev. Resp. Dis., *120*:1059–1067, 1979.
39. Holtzman, M. J., Sheller, J.R., Dimeo, M., et al.: Effect of ganglonic blockade on bronchial reactivity in atopic subjects. Am. Rev. Resp. Dis., *122*:17–24, 1980.
40. Jakeb, G. J.: Adverse effect of a cigarette smoke component, acrolein, on pulmonary antibacterial defenses and on viral-bacterial interactions in the lung. Am. Rev. Resp. Dis., *115*:33–38, 1977.
41. Jeffrey, P. K., and Reid, L. M.: The Respiratory mucous membrane. *In* Brain, J. D., Proctor, D. F., Reid, L. M., eds.: Respiratory Defense Mechanisms. N.Y., Marcel Dekker, 1977.
42. Johnson, D. A.: Ozone inactivation of human α-1-proteinase inhibitor. Am. Rev. Resp. Dis., *121*:1031–1038, 1980.
43. Karman, M. S., and Daniel, E. E.: Formation of gap junctions by treatment in vitro with potassium conductance blockers. J. Cell Biol., *78*:348–358, 1978.
44. Karman, M. S., and Daniel, E. E.: Structural and functional study of control of canine tracheal smooth muscle. Am. J. Physiol., *238*(Cell Physiol 7):C27–C33, 1980.
45. Kilburn, K. H.: A hypothesis for pulmonary clearance and its implications. Am. Rev. Resp. Dis., *98*:449–463, 1968.
46. King, M., Boileay, R., Delannois, L., et al.: Tracheal mucus in dogs exposed to SO_2. Viscoelastic changes during exposure and recovery. Physiologist, *23*:100, 1980.
47. King, M., La Engel, and Macklem, P. T.: Effect of methacholine chloride on rheology and transport

of canine tracheal mucus. J. Appl. Physiol., *46*:504–516, 1979.
48. King, M., and Macklem, P. T.: Rheological properties of microliter quantities of normal mucus. J. Appl. Physiol., *42*:797–802, 1977.
49. Knowles, M. R., Murray, G. F., Shallal, J. A., et al.: Bioelectric properties and ion transport in excised human bronchi. Physiologist,*23*:100, 1980.
50. Koefoed-Johnson,V., and Ussing, H. H.: Acta Physiol. Scand., *42*:298–308, 1958.
51. Lamb, D., and Reid, L.: Mitotic rates, goblet cell increase and histo-chemical changes in mucus in rat bronchial epithelium during exposure to sulfur dioxide. J. Pathol. Bact., *96*:97–111, 1968.
52. Lambert, P. M., and Reid, D. D.: Smoking air pollution, and bronchitis in Britain. Lancet, *42*:853–857, 1970.
53. Last, J. A., and Kaizu, T.: Mucus glycoprotein secretion by tracheal explants: Effects of pollutants. Environ. Health Perspect. *35*:131–138, 1980.
54. Lee, L. Y., Bleecker, E. R., and Nadel, J. A.: Effect of ozone on bronchomotor response to inhaled histamine aerosol in dogs. J. Appl. Physiol., *43*:626–631, 1977.
54a. Lunn, J. E., Knowelden, J., and Handyside, A. J.: Patterns of respiratory illness in Sheffield infant school children. Brit. J. Prev. Soc. Med., *21*:7, 1967.
55. McFadden, E. R.: Asthma: Airway reactivity and pathogenesis. Sem. Resp. Med., *1*:287–296, 1980.
56. Man, S. F. P., Adams, G. K., and Proctor, D. F.: Effects of temperature relative humidity and mode of breathing on canine airway secretions. J. Appl. Physiol., *46*:205–210, 1979.
57. Marin, M. G., and Zaremba, M. M.: Effect of calcium on ion transport and electrical properties of tracheal epithelium. J. Appl. Physiol., *44*:900–904, 1978.
58. Matsumura, Y., Mizuno, K., Miyamoto, T., et al.: The effects of ozone, nitrogen dioxide, and sulfur dioxide on experimentally induced allergic respiratory disorder in guinea pigs. II. Am. Rev. Resp. Dis.,*105*:250–261, 1970.
59. Matsumura, Y.: The effect of ozone, nitrogen dioxide, and sulfur dioxide on experimentally induced respiratory disorder in guinea pigs. III. Amer. Resp. Dis., *105*:262–267, 1970.
60. Matsuzaki, Y., Yhamasaki, and Said, S. I.: Vasoactive intestinal peptide: A possible transmitter of nonadrenergic relaxation of guinea pig airways. Science, *210*:1252–1253, 1980.
61. Nadel, J. A.: Neurophysiologic aspects of asthma. *In* Austen, K. F., and Linchtenstein, L. M., eds.: Asthma, Physiology, Immunopharmacology, and Treatment. New York, Academic Press, 1973.
62. Newhouse, M., Sanchis, J., and Bienenstock, J.: Lung defense mechanisms. New Engl. J. Med., *295*:990–998, 1976.
63. Nickel. National Research Council, Committee on Medical and Biological Effects of Environmental Pollutants. National Academy of Sciences, Washington, D.C., 1975.
64. Nitrogen Oxide. National Academy of Sciences, Washington, D.C., 1977.
65. Olver, R.E., Davis, B., Marin, M. G., et al.: Active transport of Na^+ and Cl^- across the canine tracheal epithelium. Am. Rev. Resp. Dis., *112*:811–815, 1975.
66. Orehek, J., Massare, J. P., Gayrard, P., et al.: Effect of short-term, low level nitrogen dioxide exposure on bronchial sensitivity of asthmatic patients. J. Clin. Invest., *57*:301–308, 1976.
67. Patterson, R. W., Harris, K. E.: The effect of cholinergic and anticholinergic agents on the primate model of asthma. J. Lab. Clin. Med., *87*:65–72, 1976.
68. Pavia, D., Thomson, M. L., Claric, S. W., et al.: Effect of lung function and mode of inhalation of penetrance of aerosols into the human lung. Thorax, *32*:194–200, 1977.
69. Pearlman, M. E., Finklea, J. E., Creason, J. P., et al.: Nitrogen dioxide and lower respiratory illness. Pediatrics, *47*:391–407, 1971.
70. Plopper, C. G., Dungworth, D. L., and Tyler, W. S.: Pulmonary lesions in rats exposed to ozone. Am. J. Pathol., *71*:375–394, 1973.
71. Ranga, V., and Kleinerman, J.: A quantitative study of ciliary injury in small airways of mice: The effects of NO_2. Exper. Lung. Res., in press.
72. Reid, L. M., and Jones, R.: Mucus membrane of respiratory epithelium. Environ. Health. Perspect., *35*:113–120, 1980.
73. Richardson, J. B.: Nerve supply to the lungs. Am. Rev. Resp. Dis., *119*:785–802, 1979.
74. Richardson, J. B.: The neural control of human tracheobronchial smooth muscle. *In* Lichtenstein, M.A., and Austen, K. F., eds.: Asthma: Physiology, Immunopharmacology and Treatment. New York, Academic Press, 1977.
75. Richardson, J. B., and Beland, J.: Non-adrenergic inhibitory nerves in human airways. J. Appl. Physiol., *41*: 764–771, 1976.
76. Richardson, J., and Bouchard, T.: Demonstration of a nonadrenergic inhibitory nervous system in the trachea of the guinea pig. J. Allergy Clin. Immunol., *56*:473–480, 1975.
77. Rinard, G. A., Rubinfeld, A. R., Brunton, L. L., et al.: Depressed cyclic AMP levels in airway smooth muscle from asthmatic dogs. Proc. Natl. Acad. Sci., *76*:1772–1776, 1979.
78. Rubinfeld, A. R., and Pain, M. C. F.: Relationship between bronchial reactivity, airway caliber, and severity of asthma. Am. Rev. Resp. Dis., *115*:381–387, 1977.
79. Russell, J. A.: Innervation of airway smooth muscle in the dog. Bull. Euro. Physiopath. Resp., *16*:663–670, 1980.
80. Sackner, M. A., Dougherty, R. D., and Chapman, E. A.: Effect of inorganic nitrate and sulfate salts on cardiopulmonary function. Am. Rev. Resp. Dis., *113*:89, 1976. Abstract.
81. Sampson, S. R., Vidruk, E. H., Bergen, D., et al.: Effects of ozone exposure on responsiveness on intrapulmonary rapidly adapting receptors to bronchoactive agents in dogs. Fed. Proc., *37*:712, 1978.
82. Satir, P.: Structural Basis of ciliary movement. Environ. Health Perspect., *35*:77–82, 1980.
83. Schoel, L. D., Oobrogorski, O. J., Mountain, O. J., et al.: Physiologic, biochemical, immunologic, and pathologic changes following ozone exposure. J. Appl. Physiol., *14*:67–80, 1959.
84. Schoettlin, C. E., and Landau, E.: Air pollution and asthmatic attacks in the Los Angeles area. Publ. Health Rep., *76*:545, 1961.
85. Shy, C. M., Creason, J. P., Pearlman, M. E., et al.: The Chattanooga school children study: Effects of community exposure to nitrogen dioxide. I. Methods, description of pollutant exposure and results of ventilatory function testing. J. Air Poll. Control Assoc., *20*(8):539, 1970. II. Incidence of Acute Res-

piratory Illness. J. Air Poll. Control Assoc., *20*(9):582, 1970.
86. Solomon, A. K.: A characterization of biological membranes by equivalent pores. J. Gen. Physiol., *51*:335–364, 1968.
87. Staehlin, L. A.: Three types of gap junctions interconnecting intestinal epithelial cells visualized by freeze-etching. Proc. Nat. Acad. Sci., *69*:1318–1332, 1972.
88. Stephens, R. J., and Parkinson, D. R.: Morphological surface changes in the terminal bronchiolar region of NO_2-exposed rat lung. Environ. Res., *6*:37, 1973.
89. Strugess, J. M., Chao, J., Wong, J., et al.: Cilia with defective radial spokes: A cause for human respiratory disease.
90. Stutts, M. J., Boucher, R. C., Bromberg, P. A., et al.: Effects of ammonium and nitrate salts on ion transport across the excised canine trachea. Toxicol. Appl. Pharm., in press.
91. Stutts, M. J., Boucher, R. C., and Gatzy, J. T.: Cd $(NO_3)_2$ and $Zn(NO_3)_2$. Effects on canine tracheal bioelectric properties. Fed. Proc., *39*:395, 1980.
92. Szentivonyi, A.: Effect of bacterial products and adrenergic blocking agents on allertic reactions. *In* Samter, M., ed.: Immunologic Diseases I. Boston, Little Brown, 1971, pp. 356–374.
93. Taylor, A. E., and Gaar, K. A., Jr.: Estimation of equivalent pore radii of pulmonary capillary and alveolar membranes. Am. J. Physiol., *218*:1133–1140, 1970.
94. Theodore, J., Robin, E. D., Gaudio, R., et al.: Transalveolar transport of large polar solutes (sucrose, insulin, and dextran). J. Appl. Physiol., *129*:989–996, 1975.
95. Ussing, H. H., and Zerahn, K.: Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta Physiol. Scand., *23*:110–127, 1951.
96. Utell, M. J., Swinburne, A. J., Hyde, R. W., et al.: Airway reactivity to nitrates in normals and asthmatics. J. Appl. Physiol.: Resp. Env. Exercise Physiol., *46*:189–196, 1979.
97. Vidruk, E. H., Hahn, H. L., Nadel, J. A., et al.: Mechanisms by which histamine stimulates rapidly adapting receptors in dog lungs. J. Appl. Physiol., *43*:397–402, 1977.
98. Vincent, N. J., Knudson, R., Leith, D. E., et al.: Factors influencing pulmonary resistance. J. Appl. Physiol.,*29*:236–243, 1970.
99. Wanner, A.: Clinical aspects of mucociliary transport. Am. Rev. Resp. Dis., *116*:73–126, 1977.
100. Watanabe, S., Frank, R., and Yokoyamn, E.: Acute effects of ozone in lungs of cats. Am. Rev. Resp. Dis., *108*:1141–1151, 1973.
100a. Wayne, W. P., Whehrle, P., and Carroll, R.: Oxident air pollution and athletic performance. J. A. M. A.,*199*:901–904, 1967.
101. Widdicombe, J. G.: Defense Mechanisms of the Respiratory System. Int. Rev. of Physiology. Respiratory Physiology II, Volume 14 Ed.by John G. Widdicombe, Baltimore, University Park Press, 1977.
102. Widdicombe, J. G.: Regulation of tracheobronchial smooth muscle. Physiol. Rev., *43*:1–16, 1963.
103. Widdicombe, J. G., Kent, D. C., and Nadel, J. A.: Mechanisms of bronchoconstriction during inhalation of dust. J. Appl. Physiol., *17*:613–616, 1962.
104. Yager, J. A., Ellman, H., and Dulfano, M. J.: Human ciliary beat frequency at three levels of the tracheobronchial tree. Am. Rev. Resp. Dis., *121*:661–666, 1980.

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